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Structure and Catalytic Mechanism of 3-Ketosteroid-Delta 4-(5 alpha)-dehydrogenase from *Rhodococcus jostii* RHA1 Genome

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SUPPLEMENTAL DATA

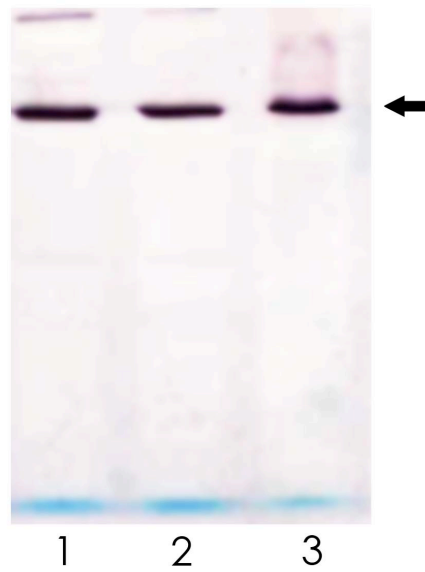
Structure and catalytic mechanism of 3-ketosteroid- Δ^4 -(5 α)-dehydrogenase (Δ^4 -(5 α)-KSTD)
from *Rhodococcus jostii* RHA1*

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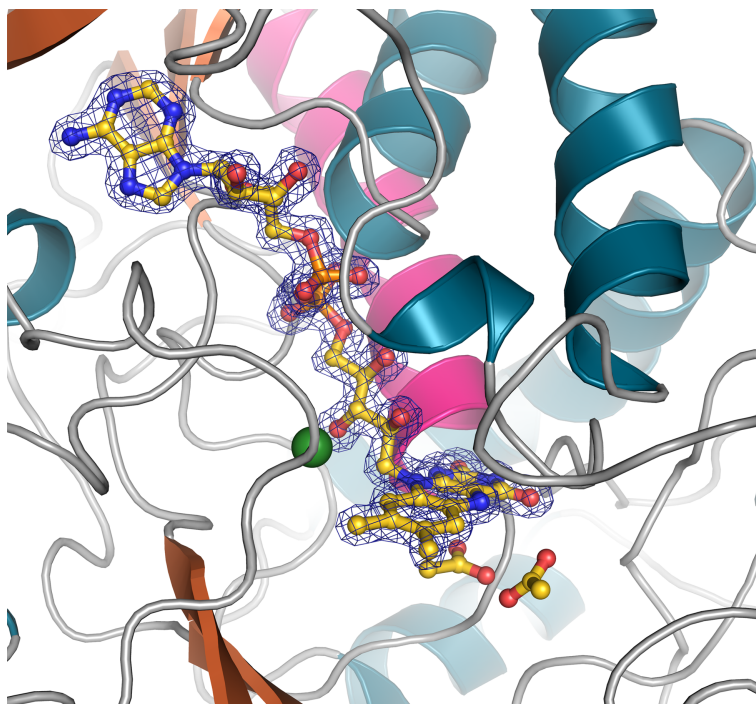
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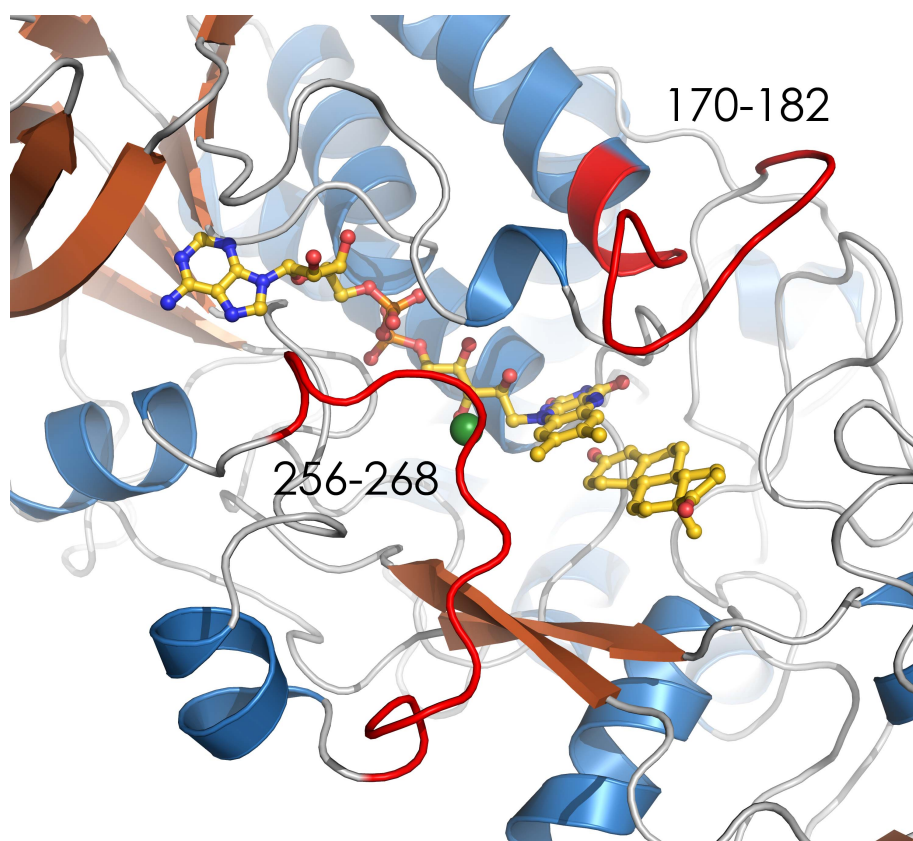
Supplemental Figure 1

Activity-stained native PAGE gel of $\Delta 4$ -(5 α)-KSTDs from *R. erythropolis* SQ1 (lane 1), *R. rhodochrous* DSM43269 (lane 2) and *R. jostii* RHA1 (lane 3). $\Delta 4$ -(5 α)-KSTD activity was visualized (black arrow) by incubating a native PAGE gel loaded with cell-free extracts (30-50 μ g of protein) in a Tris-HCl buffer containing the electron acceptors phenazine methosulphate and nitro blue tetrazolium, as well as the substrate 1-(5 α)-androstene-3,17-dione (1-(5 α)-AD).



Supplemental Figure 2

FAD binding in $\Delta 4-(5\alpha)$ -KSTD. The electron density around the FAD is shown at 1σ . The C-terminal helix, which is thought to provide a helix dipole moment important for the reaction, is shown in pink. The FAD and acetate molecules are yellow and the chloride ion is shown in green.



Supplemental Figure 3

Two loops (residues 170-182 and 256-268, shown in red) close to the active site. Both show less well-defined electron density, which suggests flexibility of these loops. The FAD and 4AD are shown in yellow and the chloride ion in green.

Supplemental Table I – Sequences of sense primers for site-directed mutagenesis
The mutated nucleotides and corresponding amino acids are printed in bold.

Mutation	Primer										
W136A	tgg W	ggc G	gaa E	ccc P	gga G	gcg A	gaa E	ccc P	ccg P	ttc F	gac D
W136F	gg	ggc G	gaa E	ccc P	gga G	ttc F	gaa E	ccc P	ccg P	ttc F	gac D
Y319F	gtt V	ccg P	gag E	gac D	acc T	ttc F	tcc S	ggg G	cgg R	atc I	gg
Y466A	gtg V	tgc C	gcg A	ggc G	ggc G	gcc A	gcc A	agc S	ggc G	acc T	tcg S
Y466F	gtg V	tgc C	gcg A	ggc G	ggc G	ttc F	gcc A	agc S	ggc G	acc T	tcg S
S468T	gcg A	ggc G	ggc G	tac Y	gcc A	acc T	ggc G	acc T	tcg S	ctc L	gg